BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **230,** 347–350 (1997)
ARTICLE NO. RC965958

Fine Mapping of Five Human Skeletal Muscle Genes: Alpha-Tropomyosin, Beta-Tropomyosin, Troponin-I Slow-Twitch, Troponin-I Fast-Twitch, and Troponin-C Fast

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Received December 2, 1996

In this paper the chromosomal localization of the human skeletal muscle genes Troponin-I slow-twitch (TNNI1), Troponin-I fast-twitch (TNNI2), and Troponin-C fast (TNNC2) and the refinement of the position for alpha-Tropomyosin (TPM1) and beta-Tropomyosin (TPM2) are reported. By radiation hybrid mapping, TPM1 was assigned to chromosome 15q22.1, TPM2 to chromosome 9p13.2-p13.1, TNNI1 to chromosome 1q31.3, TNNI2 to chromosome 11p15.5, and TNNC2 to chromosome 20q12-q13.11. The genomic distribution of these genes is discussed, with particular emphasis on the cluster organization of the Troponin genes.

Radiation hybrid (RH) mapping is a powerful method for constructing long-range maps of mammalian chromosomes (1). The number of human genes mapped by this technique is progressively increasing. In particular, RH mapping is one of most efficient approaches (2) to the fine localization of human genes, whose sequence is already known. Our lab is engaged in the identification and mapping of ESTs from human skeletal muscle (3) (4). In the course of this work, we discovered with surprise that some important genes expressed in the skeletal muscle (i.e. alpha-Tropomyosin, beta-Tropomyosin, Troponin-I slow-twitch, Troponin-I fast-twitch and Troponin-C fast) were still unmapped or imprecisely mapped, although their sequence was already available in Genbank.

Since the ESTs obtained in our lab correspond to 3′-end portion of skeletal muscle mRNAs (3) and the untranslated regions are poorly conserved, there is a good probability that the designed primers will selectively amplify a distinct human fragment. Moreover, related members of a gene family can be very similar in the coding region but are normally considerably different in the less conserved 3′ untranslated part. Therefore, RH mapping by using 3′ end primers is likely to identify unambiguously the location of the selected gene (5) (6). We decide then to design PCR primers from the 3′ESTs corresponding to the genes of interest, or from the 3′ end of the sequence available in Genbank, in order to map them by RH mapping. The results are reported in the present paper, with particular reference to their possible role as candidate genes for human disease.

MATERIALS AND METHODS

Primer designing. Oligonucleotides for PCR were selected by PRIMER3 software (7) from EST sequences present in our database (http://eos.bio.unipd.it/), except for TPM1 primers, which were derived from the Genbank sequence M19715. TPM2, TNNI1, TNNI2 and TNNC2 primer pairs were designed on the ESTs 1520, 556, 4323 and 451, respectively, corresponding to the EMBL codes: F17006, F16078, F19718, F15975.

We have excluded as target sequences for the primer selection the regions very close (about 20 bp) to the polyA tract, where signals of polyadenylation could be present.

Given the excessive shortness of the TNNI2 untranslated region, we designed primers located between putative exon-exon junctions in the cDNA sequence, using the RNASPL program (8). The selected primers were:

Primer	Expected PCR product size (bp)	Annealing temperature (°C)
TPM1-FOR: 5′ gtggggaaaacacatacaaaaag 3′ TPM1-REV: 5′ cttcctgttgactctatcattgg 3′	189	60
TPM2-FOR: 5′ agtctatgcccagaagatgaagt 3′ TPM2-REV: 5′ gataggtaaaggatgaagccagt 3′	266	62.5
TNNI1-FOR: 5′ agtgctgaagacctcaccct 3′ TNN-1-REV: 5′ tcctcctcctccattgttac 3′	152	58
TNNI2-FOR: 5′ aggtcaagaaggaggacacag 3′ TNNI2-REV: 5′ gggtgcatctccctagtatgt 3′	190	65
TNNC2-FOR: 5′ cgacaggaatgcagacggctacatc 3′ TNNC2-REV: 5′ tggggacccggcagggcggagtctc 3′	247	54

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0006-291X/97 $25.00

Radiation hybrid mapping. The radiation hybrid mapping was performed by the Genebridge 4 whole-genome Radiation Hybrid Panel (Research Genetics, Huntsville, Al., U.S.A.) consisting of 93 genomic DNAs from the same number of human-on-hamster somatic cell lines, plus the two control DNAs (HFL donor and A23 recipient) (9).

Twenty-five ng of genomic DNA was used for amplification in 10 μl of PCR buffer (16.6 mM (NH4)2SO4; 67 mM Tris-HCl pH 8.3; 0.01 % Tween-20; 1.5 mM MgCl2) containing 800 nM of each of the forward and reverse primers, 0.2 units of DNA polymerase (RTB polymerase; Bioline, Italy) and 25 μM of each of the four dNTPs. The mix was covered with 5 μl of mineral oil.

Cycling conditions were: 1 min and 15 sec at 94 °C, followed by 35 cycles of 15 sec at 94 °C, 25 sec at the working annealing temperature and 30 sec at 72 °C, and a final extension step for 1 min and 30 sec at 72 °C (PTC-225 Peltier Thermal Cycler, MJ Research).

The PCR reactions were mixed with 5 μl of loading buffer (30 % glycerol; 0.25 % Orange G; 25 mM EDTA) and separated on 2.5 % agarose gel in TAE buffer (40 mM Tris-acetate and 1 mM EDTA) stained with ethidium bromide. The gel was electrophoresed in the same buffer at 100 Volts for 45 min.

The screening results were processed by the RHMAPPER software program (10) at the Whitehead Institute/MIT Center for Genome Research (Cambridge, Mass., U.S.A.).

One centi Ray (cR) corresponds to a one percent frequency of breakage between the involved markers, at a given dose of radiation, used for the chromosome fragmentation. In this study the panel of hybrids was obtained with a dosage of 3000 rad. Approximately, one cR corresponds to 300 kb (11).

The cytological localization of each gene to be mapped was deduced from the framework of flanking markers, obtained from Location Data Base (LDB) (12).

DNA sequencing. The TNNI2 PCR product was purified using the QIAquick Spin column (QIAGEN) and sequenced using fluorescent dideoxy terminators, the same amplification primers and the Taq FS, on a ABI 377 automated DNA sequencer, following ABI protocols.

RESULTS AND DISCUSSION

PCR primers specific for the 3′ untranslated region of the human TPM1, TPM2, TNNI1 and TNNC2 cDNAs (and for exonic regions of TNNI2) were designed and used to PCR amplify the human / hamster somatic cell hybrid DNAs (Genebridge 4 whole-genome Radiation Hybrid Panel). The expected size for all the PCR products was confirmed by the electrophoretic separation on polyacrylamide gel, except for TNNI2. In this case, DNA sequencing of the amplified product (437 bp) revealed the presence of a 247 bp intron, placed between the sixth and the seventh base downstream the forward primer (corresponding to nucleotides 481 and 482 of the Genbank sequence L21715).

The retention scores, obtained on the Genebridge 4 Panel for the genes under investigation, are shown in Table 1.

According to multipoint linkage, the alpha-Tropomyosin (TPM1) gene was mapped on chromosome 15q22.1, with a lod score >21 (Fig.1). The gene is 2 cR from D15S1196 (WI-9443) (at 64.339 Mb in the physical map) and 0 cR from D15S159 (WI-5241), which maps at 63.860 Mb from the p-telomere. TPM1 was previously mapped by FISH on 15q22 band (13). There is a considerable difference between our localization, very close to D15S159, and that reported for TPM1 on the LDB map, which placed it about 15 Mb from this marker on the telomeric side. This discrepancy is essentially due to the relative imprecision of the previous mapping, obtained by FISH.

The beta-Tropomyosin gene (TPM2), previously localized by FISH on 9p13 (14), was placed, with a lod score >19, between 9p13.2 and 9p13.1, at 3.87 cR from AFM326VD1 (at 37.302 Mb in the physical map) and 2.6 cR from D9S1705 (WI-7534), at 45.298 Mb (Fig. 2). Interestingly, two skeletal muscle disorders, the arthrogryposis multiplex congenita, an inherited amyoplasia (AMCD1; OMIM 108120), and the inclusion body myopathy (IBM2; OMIM 601073) map in the same region (15) (16).

The Troponin-I slow-twitch (TNNI1) gene, previously assigned to chromosome 1q12-qter (17), in this study mapped to 1q31.3, 1.5 cR from D1S2419 (WI-9272) (at 209.573 Mb) and 3 cR from D1S2097 (WI-4586), which is placed at 210.598 Mb from the p-telomere (Fig.3). In this case, like the others, the lod score was significantly high (> 17). It is interesting to note that the primers for D1S2419 were originally obtained from the TNNT2 sequence (cardiac Troponin-T; Genbank X74819) (18) (11). Therefore, the two genes TNNI1 and TNNT2 appear to be closely linked, being less than 1 Mb apart.

The map position of Troponin-I fast-twitch (TNNI2) gene was unknown. Our study placed this gene on chromosome 11p15.5, with a lod score > 15. The gene is

TABLE 1

Gene	Retention profile
TPM1	10010210011000210000000000000001102000200211100100010000001000000110000101010100001201110101111
TPM2	01000010000001000110001000000010010000100010110011202001110111001001000001221000200010100001 2
TNNI1	00001000100010010020000011000000100000101000000000100000002000100100100101000000011000000100 0
TNNI2	02200010002210000010100201211010010002010121000200110010001100000011222100000001110110000112 0
TNNC2	10100100000001111110102122100110022120021110000000001100111100021210000011011100000021100202 0

Note. Retention scores for the genes on the Genebridge4 Radiation Hybrid Panel. A hybrid scored 1 indicates presence of the sequence in that hybrid. A hybrid scored 0 indicates absence of the sequence. Unknown/uncertain data are indicated with 2's. The hybrids in the data vector are arranged according to the official Genebridge4 order.

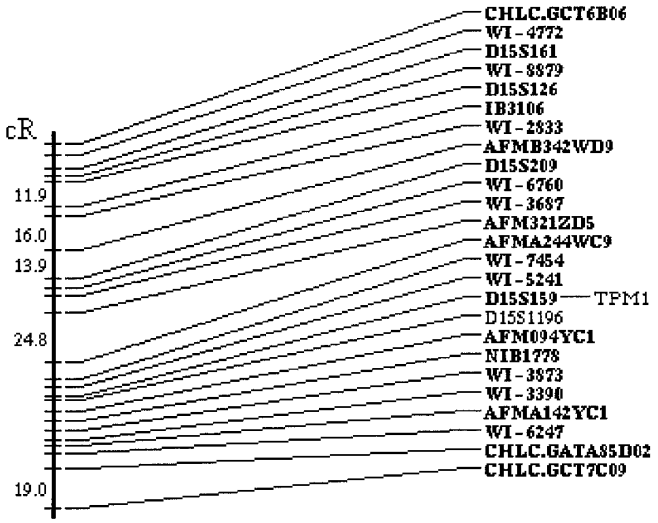

FIG. 1. Chromosome 15 radiation hybrid framework map with TPM1 gene placed on the q22.1band.

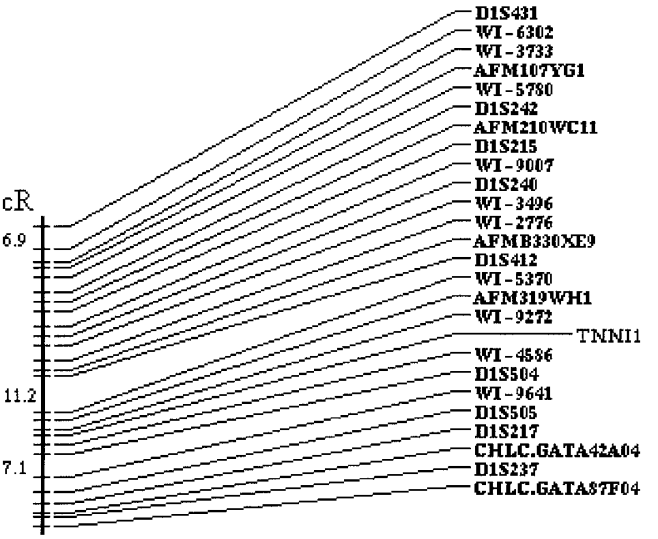

FIG. 3. Chromosome 1 radiation hybrid framework map with TNNI1 gene placed on the q31.3 band.

located very close to the telomere, at 0 cR from D11S4033 (WI-4920) and at 3.2 cR from D11S4393 (WI-1421), which is only at 0.073 Mb in the physical map (Fig. 4). This result is in agreement with the observation that the mouse Tnni2 gene maps in a region of chromosome 7 which is syntenic with portions of the human chromosome 11 (19).

Finally, the gene for Troponin-C fast-twitch (TNNC2) was assigned by our study to chromosome 20q12-q13.11 (Fig. 5), at 4.81 cR from D20S481 (CHLC.GATA47F05) which is placed at 52.627 Mb on the physical map, and 2.4 cR from NIB1800, which lies at 53.221 Mb. No disease genes have yet been mapped so far in the regions corresponding to the localization of genes TNNI1, TNNI2 and TNNC2.

The results obtained in the present study provide additional support to the hypothesis that troponin genes are organized in clusters. The first cluster was described on chromosome 19q13.3-q13.4, where TNNT1 and TNNC1 were mapped and where TNNI3 is possibly located (20) (21) (22). Our study demonstrated the possible existence of two additional clusters, one on chromosome 1q31.3, where TNNI1 maps close to TNNT2, and the other on chromosome 11p15.5., where TNNI2 maps in proximity of TNNT3 (23). On the other hand, the organization of the troponin genes in clusters was expected, since the members of this gene family should be coordinately activated during muscle development and differentiation.

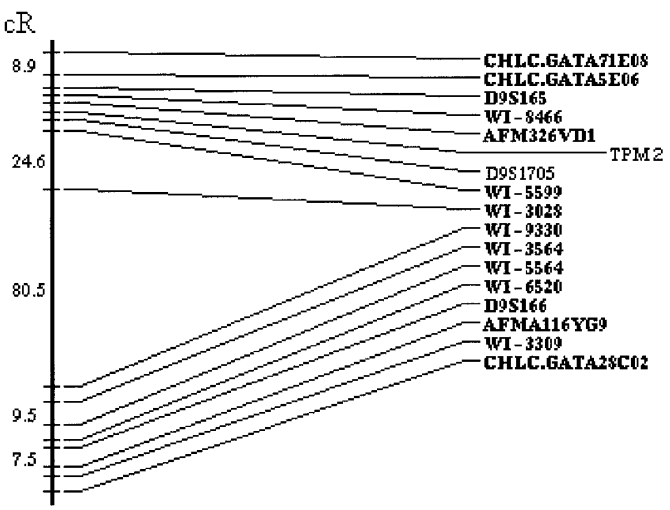

FIG. 2. Chromosome 9 radiation hybrid framework map with TPM2 gene placed on the p13.2–p13.1 region.

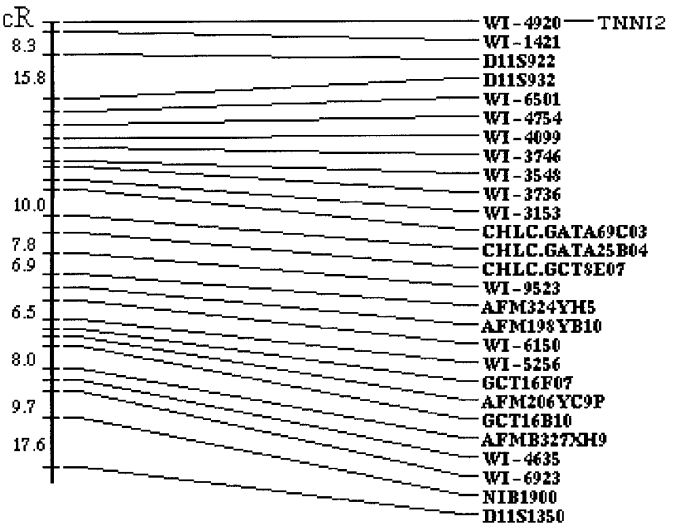

FIG. 4. Chromosome 11 radiation hybrid framework map with TNNI2 gene placed on the p15.5 band.

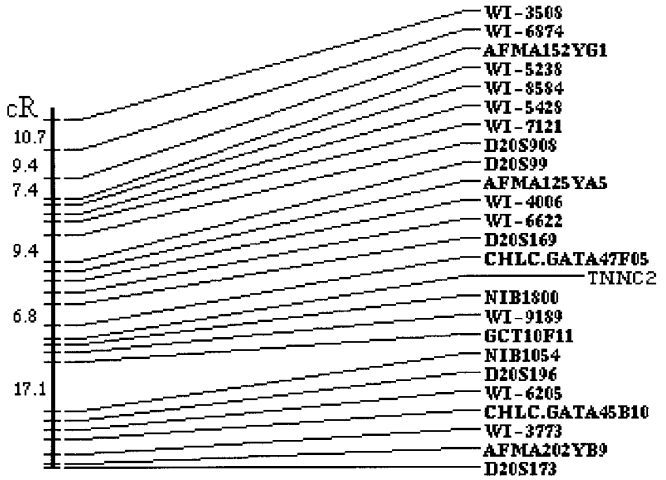

FIG. 5. Chromosome 20 radiation hybrid framework map with TNNC2 gene placed on the q12–q13.11 region.

ACKNOWLEDGMENTS

This work was supported by Comitato Promotore Telethon, Italy; Project B 30. OMIM stands for Online Mendelian Inheritance in Man and is maintained by the Center for Medical Genetics, Johns Hopkins University, Baltimore, and by the National Centre for Biotechnology Information, National Library of Medicine, Bethesda, MD.

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